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BIOLOGICAL BULLETIN

THE EFFECT UPON DEVELOPING EGGS OF EXTRACTS OF EMBRYOS OF THE SAME SPECIES.[1]

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INTRODUCTION.

The investigations described in this report are an attempt to isolate formative substances from the early embryological stages in the developing egg of *Arbacia punctulata.* Upon the hypothesis of formative stuffs, egg extracts should show the presence of such materials, if under the conditions of the experiment they are isolable. Since development is conceived of as a series of exceedingly complex reactions involving both chemical and physical factors, the formative substances present in the fertilized egg must be the substances which begin this long sequence of reactions. If by their action and interaction, other and possibly more complex substances are synthesized, then at a given stage in development we should expect to find in the extracts of the larvæ the formative substances of that period, provided of course they go into solution in the solvents used. If such is the case, it seems decidedly important to find out whether by treatment of fertilized eggs with extracts of larvæ at a given stage, it is possible to accelerate the development of the eggs when they reach

[1] Contribution from the Zoölogy Department, Oberlin College and the Marine Biological Laboratory.

that specified stage. The cell walls of the developing eggs presumably must be permeable to the formative stuffs present in the solution, if any effect is to be registered.

The whole of the work was done at the Marine Biological Laboratory at Wood's Hole during the summer of 1921. The writer is indebted to Professor C. G. Rogers for suggesting the problem and for his criticism of the plan of work.

Methods and Materials.

The animals used in these experiments were the starfish, *Asterias forbesii,* and the sea-urchin, *Arbacia punctulata.* The eggs and sperm of both were secured by shaking the ovaries or testes into a dish of sea water. The starfish eggs were filtered through silk bolting cloth to remove portions of the ovarian tissue. The animals were first washed under a stream of fresh water and all precautions were taken to cleanse thoroughly the glassware and instruments used.

By "egg extract" is meant a suspension of crushed eggs in sea water or other specified solvent. The eggs were centrifuged, the fluid decanted, and the eggs ground up with fine sea sand, free from impurities, and thoroughly washed by the solvent used.

In some experiments the eggs were crushed between glass plates, but this method was not satisfactory. No uncrushed eggs were present in the solutions to invalidate the percentages. Extracts of larvæ were made up in the same manner. To maintain as uniform concentration as possible, a definite proportion of eggs to solvent was kept (1 c.c. eggs to 5 c.c. solvent) but the concentration was probably lowered by the retention of some of the protoplasmic fragments in the sand.

The term "blastula water" or "gastrula water," for example, is understood to mean water in which these larvæ have stood up to that stage in their development. Both the eggs and sperm in any one experiment were derived from one individual each whenever possible. The sperm suspension used was concentrated enough to cause 100 per cent. fertilization.

Data of the exact time of the 2-, 4- and 8-cell cleavages chiefly are noted. Although it is difficult to say just when, in terms of

actual minutes after fertilization, the late gastrula becomes a pluteus for instance, by careful comparison of the culture with the control one can observe any differences in their stages of development.

Two methods of comparing rates of cleavage were employed: the time elapsing between insemination and the first 2-, 4- or 8-cell stage, or the period between insemination and the time the culture showed a 50 per cent. development of a given stage. For obvious reasons the former method was used for the most part, only the time required for making a single observation elapsing between the readings, and, in order that there might be no errors introduced merely by the arrangement of the culture dishes, the order of readings was frequently reversed. Equal quantities of eggs from the same suspension were used for each culture to insure an equal opportunity for oxidation and presumably then an equal concentration of CO_2 in the water. The cultures were kept in flat-bottomed finger bowls and were stirred frequently. During the season the temperature of the room varied from 21° to 23.5° C.; the variation in the temperature of the sea water in the circulation as recorded each day was found to be from 18.1° C. to 20.5° C. The pH of the sea water was tested each day and was found to be very constant—8.2 or at a few times 8.0 when thymol blue was the indicator used. Whenever the quantity of extract was sufficient to admit of its being tested, the pH was found to be 8.0 or slightly more acid. It did not appear that there was a characteristic difference between the degrees of acidity noted in the extracts made of larvæ in different stages of development.

A new supply of animals was brought into the laboratory each day. These were kept in an aquarium placed on a cement water table with a stream of fresh water running into the aquarium constantly.

The basis of comparison between a normal and a retarded culture in all experiments was (1) rate of development, (2) size, (3) vigor (rate of movement), (4) longevity.

Sample tables are not given for the experiments on the starfish because the rates obtained for both the earlier and late stages were so varied that no definite conclusions can be fairly drawn from them. These variations were doubtless to be explained in part

at least by the fact that toward the end of the breeding season the physiological condition of the eggs was far from uniform. In general it was found that the starfish cultures containing extract showed a slight retardation, a higher degree of cytolysis, and a greater tendency to stop either at the blastula or the gastrula stage.

All the extracts used in these investigations upon *Asterias* eggs were made in a solution of sea water.

The Effects of Embryological Extracts on the Early Stages of the Sea-Urchin Egg.

The following experiments were all performed on the sea-urchin, *Arbacia punctulata,* to find out if the extracts of various larval stages have any effect on developing eggs of the same species. The eggs of *Arbacia* while more stable, and therefore less desirable than those of *Asterias,* can be obtained in convenient quantities for use in making extracts.

About sixty series of experiments were performed. The method was strictly comparative. It will be noted that the tables show, not only the effect of a given extract, but its effect in varying concentrations as well.

Particular emphasis is laid on the fact that the experiments given in outline in the table are type experiments. Each experiment outlined is one of several very similar experiments unless otherwise stated and is taken as fairly representative of the results obtained in all the experiments of that series, the others of which are omitted to avoid unnecessary and tedious repetition.

Series 15.

Table I.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	50 c.c.	25 c.c.	Gastrula	18.2° C.	8.2	8.0	44	92	117
A'....	50 c.c.	25 c.c.	"	"	"	"	49	97	114
B.....	75 c.c.			"	"		48	75	98
B'....	75 c.c.			"	"		47	71	96

Note.—In this particular experiment the figures given under cleavage rate are in terms of minutes which elapsed between insemination and the appearance of the first 2-, 4- or 8-cell stage. It should be noted that the amount of extract used in this series was relatively large. (pH significance discussed later in paper.)

Later Development and Fate.—The eggs in cultures *A* and *A'* stopped at the 128–256-cell stage, and a very general cytolysis occurred.

The eggs in *B* and *B'* developed into vigorous normal plutei.

Conclusion.—The table shows that there was a definite retardation at the second and third cleavages in the cultures containing extract, and a complete arrest of development at the beginning of the early non-motile blastula stage.

Series 17.

TABLE II.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	25 c.c.	25 c.c.	Blastula Water	18.4° C.	8.2	8.0	50	84	89
A'....	50 c.c.			"	"		50	67	84
B.....	40 c.c.	10 c.c.	Motile Blastula	"	"	"	51	68	89
B'....	50 c.c.			"	"		49	66	83
C.....	45 c.c.	5 c.c.	"	"	"	"	51	77	81
C'....	50 c.c.			"	"		51	67	83
D....		50 c.c.	Blastula Water	"			68	106	138
D'....				"	"		48	65	84

Note.—In this experiment the figures given under cleavage rate are in terms of minutes which elapsed between insemination and the presence in the culture of fifty per cent. of the stage indicated.

Later Development and Fate.—Development in cultures *B, C,* and to a slight degree in *A,* was definitely retarded, especially between the gastrula and early pluteus stages. In the great majority of cases the gastrulæ did not become plutei at all. The eggs in culture *D* were very much retarded; in fact, few progressed beyond the blastula stage, due probably to lack of oxygen in the water, and possibly to a high concentration of carbon dioxide. Many of the eggs in the controls developed into plutei.

Conclusion.—The table shows a very slight retardation in the early cleavage rate of *A*, *B* and *C*, and a greater amount of retardation in the later stages, especially at the early gastrula stage. *D* was very markedly retarded.

A very unusual proportion of exogastrulæ was found in all the cultures. There was even a small proportion in the controls. The reason for this is unknown. It happened that this particular series of experiments was carried on during the hottest days of the summer, but the bowls were kept in the water table, and the temperature of the room was not above 23° C. Later experiments failed to show an equally high percentage of exogastrulæ, although they were carried on under conditions as nearly identical as possible. The only explanation to be offered is that there may have been some variation in the eggs of the particular female used which caused them to evaginate instead of invaginate.

The question of whether these abnormal cultures of exogastrulæ could possibly be influenced to develop normally if extract of larvæ at the same stage was added came up at this point. Of course it is obviously improbable that in making the extract one would at the first trial secure larvæ at the identical point of development of those in the culture. The eggs in the culture were, however, centrifuged, the stale water decanted, and fresh water added. Numerous trials of extracts made up of embryos at different points in the same general developmental stage, and in preceding and succeeding stages were used. In no case did the exogastrulæ proceed with their development.

Series 32.

TABLE III.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	50 c.c.	15 c.c.	Pluteus	18.6° C.	8.2	8.0	62	118	131
A'....	65 c.c.		"	"	"		55	79	98
B.....	50 c.c.	10 c.c.	"	"	"	"	62	96	116
B'....	60 c.c.		"	"	"		54	77	99
C.....	50 c.c.	5 c.c.	"	"	"	"	57	79	120
C'....	55 c.c.		"	"	"		56	78	96
D.....	30 c.c.	20 c.c.	Pluteus Water	"	"		57	84	104
D'....	50 c.c.		"	"	"		56	80	98

Note.—The cleavage rate is given in terms of minutes between insemination and a fifty per cent. development of the specified stage.

Later Development and Fate.—The controls developed faster and more normally than did the other cultures, which were all retarded in the early cleavage stages and at the non-motile blastula stage. All the cultures finally developed into gastrulæ. Some exogastrulæ were found in all the cultures containing extract. No development beyond the late gastrula or very early pluteus stage was observed in the cultures containing extract.

The controls showed normal development.

Conclusion.—Definite retardation was noted in the cultures containing extract and total arrest at the late gastrula or early pluteus stages.

Series 26.

TABLE IV.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A.....	40 c.c.	12 c.c.	Water fr. 128–256 cell stage	18.6° C.	8.2	8.0	44	77	91
A	52 c.c.			"	"		36	70	86
B.....	50 c.c.	5 drops	128–256 cell stage	"	"	"	44	72	85
B'....	51 c.c.			"	"		39	70	84
C.....	50 c.c.	10 c.c.	"	"	"	"	49	76	93
C'....	60 c.c.			"	"		37	68	81

Note.—Under "cleavage rate" is noted the number of minutes which elapsed between insemination and the appearance of the first 2-, 4- or 8-cell stage.

Later Development and Fate.—Development to the gastrula stage was nearly parallel in all the cultures. *A* did not develop into very vigorous plutei; *B* showed plutei a trifle smaller than normal, while in *C* were found plutei, but many abnormal types. All the controls had normal plutei.

Conclusion.—Slight retardation in cultures containing the extract or the "water."

Several experiments were performed using instead of extracts, water in which eggs had developed. As above specified, "blastula water" is understood to mean water in which eggs have developed to the blastula stage. Details of these experiments are not given because the results do not differ in any way from those obtained by the use of such water as indicated in the above tables. If a relatively large amount of blastula water, *i.e.*, 15–20 c.c. to 50 c.c. of sea water is used, a very slight retardation is noticed when the culture is compared with the control. If only very small amounts of this "water" are employed no perceptible retardation occurs. Since these results seemed to indicate that possibly the retardation in the case of the eggs developing in the blastula water, for example, was due simply to an insufficient supply of O in the water together with increased acidity, boiled sea water was substituted for the blastula water. A very slight retardation was observed—not so evident by any means as that seen in the controls employing blastula water, and quite within the limits of experimental error. This decrease in the degree of retardation, however, may have been due in part, at least, to a decrease in the number of bacteria present in the cultures made up with the boiled sea water.

Since in all the experiments it was quite evident that there was a shorter length of life in the cultures in which extracts were used, an experiment was made using extracts of suspensions which had been boiled previous to being centrifuged, and comparisons drawn

Series 36.

TABLE V.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	100 c.c.	30 c.c.	Blastula (boiled)	19.7° C.	8.2		55	100	—
A'....	130 c.c.			"	"		45	99	—
B.....	100 c.c.	10 c.c.	"	"	"		50	95	—
C.....	100 c.c.	5 c.c.	"	"	"		49	81	—
D.....	100 c.c.	30 c.c.	Blastula Water	"	"		52	82	—
E.....	100 c.c.	10 c.c.	"	"	"		51	80	—
F.....	100 c.c.	5 c.c.	"	"	"		48	78	—
F'....	—	100 c.c.	"	"	"		—	—	—

with the results obtained by the use of unboiled extracts. Boiling the suspension to be used for extract was done not only to destroy bacteria, but also to take into account the bare possibility that substances in the larvæ which were evidently not soluble in cold sea water might go into solution in hot water.

Note.—Cleavage rate given in terms of minutes which elapsed between insemination and the appearance of the first 2-, or 4-cell stage.

Later Development and Fate.—Development in all the cultures was nearly parallel through the blastula stage except in *F'*, in which the eggs failed to segment at all. From the blastula to the pluteus stage the cultures containing extract were very slightly retarded as compared with the control *A'*. There was also a very slight retardation in the cultures containing the specified amounts of blastula water. In all cases, as usual, the retardation resulted in the production of plutei varying slightly from the normal in many respects, but yet producing no one definite type of abnormality.

Boiled extracts of gastrula and early plutei were made also, but the results obtained were entirely similar to those indicated above.

Conclusion.—Boiling the extract appears to reduce, if not to eliminate entirely the amount of retardation in the cultures.

Two other methods of making extract in a solution of sea water were tried, since it is obvious that if the extracts were made in a solution of the same osmotic pressure as that in which the eggs developed, that is, sea water, the problem would be much simplified.

The first variation in the method of making the extract was as follows: The eggs were centrifuged, and after the supernatant fluid had been decanted they were placed in a celloidin tube containing distilled water and set in a beaker of sea water. The eggs swelled and burst, presumably setting free substances which, if soluble in water, would dialyze through the celloidin tube into the sea water in the beaker. Extract made in this way was used, but the results obtained were not positive enough to be of value without further experiment. The "double sea water" method was also employed in one series of experiments with equally incon-

clusive results. No results were obtained, however, which were in any way contradictory to those previously noted.

Series 56.

An experiment was made, using motile blastulæ for extract, but instead of sea water, distilled water was employed, and the crushed eggs left over night (12 hours) in the distilled water before the extract was added to the cultures.

TABLE VI.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c.	5 c.c.	Motile blastula	20.3° C.	8.2
A'.....	50 c.c. sea water 5 c.c. distilled water	—		"	"
B.....	50 c.c.	3 c.c.	"	"	"
B'.....	50 c.c. sea water 3 c.c. distilled water	—			
C.....	50 c.c.	1 c.c.	"	"	"
C'.....	50 c.c. sea water 1 c.c. distilled water	—		"	"

Later Development and Fate.—All the cultures and the controls appeared to reach the 128–256-cell stage at approximately the same time. A reading made four hours and fifteen minutes later showed *A* retarded, non-motile blastulæ; *A'*—blastulæ—a few motile. In *B* and *B'*, there were blastulæ, motile in both, but more vigorous in *B'*. No difference appeared at this stage in *C* and *C'*, in both of which the blastulæ were motile.

In the readings made on the following day, it appeared that *A* had stopped at the non-motile blastula stage. In *A'* were found vigorous gastrulæ. In *B* there were only a very few blastulæ which were moving while *B'* showed very vigorous late gastrulæ. No difference was noted between cultures *C* and *C'*. Both of these showed vigorous late gastrulæ.

Conclusion.—The cultures containing an appreciable amount of extract exhibited a retardation at the early non-motile blastula stage—an effect which was carried over into the later stages also.

Although the results tabulated above seemed to be practically conclusive, the question arose as to whether it might not be inter-

esting as well as worth-while to use extracts made up in solvents other than sea water. Experiments made with such extracts should be of use as a check upon the other experiments. Chambers has contended that when a cell is broken into fragments by mechanical means, the small pieces, because of surface tension, usually become spherical in shape, and form a new surface film. Perhaps, then, by breaking down such a possible film by the use of solvents other than water, more of the "formative stuffs" might go into solution.

Series 47.

The extract in this experiment was made by placing the crushed embryos, motile blastulæ, in faintly acidified distilled water. The extract was allowed to stand for twelve hours before it was used. The acidified water was made by adding four drops of glacial acetic acid to 100 c.c. of distilled water.

The controls were, of course, made by adding equal quantities of the acidified water to the sea water when certain quantities of extract were used in the cultures.

TABLE VII.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	25 c.c.	5 c.c.	Gastrula	19.6° C.	8.2
A'.....	25 c.c. sea water			"	"
	5 c.c. acid water	—			
B.....	25 c.c.	3 c.c.	"	"	"
B'.....	25 c.c. sea water			"	"
	3 c.c. acid water	—			
C.....	25 c.c.	1 c.c.	"	"	"
C'.....	25 c.c. sea water			"	"
	1 c.c. acid water	—			
D.....	50 c.c.	5 c.c.	"	"	"
D'.....	50 c.c. sea water			"	"
	5 c.c. acid water	—			

Later Development and Fate.—Development in the cultures and controls was very nearly parallel until the blastula stage was reached. At this point, however, a very decided retardation in all the cultures containing extract was noted.

C was only very slightly retarded, however, at the blastula stage, and reached the pluteus stage very shortly after *C'*.

B and *A* showed many extreme modifications of form. Only a few plutei developed. *A'* and *B'* showed plutei better developed. In all the other cultures with and without extract, plutei were found, although many variations from the normal type were noted.

A separate control of eggs in sea water was kept. This showed a general retardation of development in all the cultures and controls containing even a small per cent. of acid.

Conclusion.—In the cultures containing extract an added retardation was shown when comparison with the controls was made. This retardation occurred chiefly at the early non-motile blastula stage.

Series 59.

In this experiment the extract was made of ciliated blastulæ in a solution of ether and water (1 part of ether to 2 parts of water, distilled). The water was used merely because it is very difficult to wash the extract from the sand by ether. Since water and ether are only very slightly miscible, the extract had to be shaken very vigorously before it was used.

TABLE VIII.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c.	5 c.c.	Ciliated blastula	19.7° C.	8.2
A'.....	50 c.c. sea water			"	"
	5 c.c. of ether-water mixture	—			
B.....	50 c.c.	2 c.c.	"	"	"
B'.....	50 c.c. sea water			"	"
	2 c.c. of ether-water mixture	—			
C.....	50 c.c.	1 c.c.	"	"	"
C'.....	50 c.c. sea water			"	"
	1 c.c. of ether-water mixture	—			

Later Development and Fate.—*A* and *A'* showed very irregular cleavage from the first. Development in *B* and *B'*, *C* and *C'* was very nearly parallel through the motile blastula stage, but *A*, *B*, and *C* showed retardation in their development from the blastula stage to the pluteus stage. When the cultures were ex-

amined later, *C'* had many normal plutei; *C* had just a few small plutei, swimming feebly. *B* and *A* were retarded in their development, with fewer and smaller plutei than those in *B'* and *A'*. *A* showed by far the largest number of abnormal forms.

Conclusion.—A retardation was noted especially between the blastula and the pluteus stages in the etherial extract cultures.

Series 45.

The extract used in this experiment was made by soaking the embryos (motile blastulæ) after the usual crushing process, in a solution of one part of acetone in one part of distilled water, for 12 hours. This same solution was used in preparing the controls, and was always shaken vigorously before using.

TABLE IX.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c. sea water 5 c.c. 50 per cent. acetone	—		19.9° C.	8.2
A'.....	50 c.c.	5 c.c.	Motile blastula in 50 per cent. acetone	"	"
B.....	50 c.c. sea water 2 c.c. 50 per cent. acetone	—		"	"
B'.....	50 c.c.	2 c.c.	"	"	"
C.....	50 c.c. sea water 1 c.c. 50 per cent. acetone	—		"	"
C'.....	50 c.c.	1 c.c.	"	"	"

Later Development and Fate.—Development in the cultures and the controls appeared to be very nearly parallel until the early non-motile blastula stage. At that point in development blastulæ, some swimming vigorously, were found in *A* while in *A'* most of the blastulæ were non-motile, although a very few were moving feebly. In *B* and *B'*, *C* and *C'* there was also an evident retardation, although it was not so great as that registered in the *A* and *A'* cultures.

On the following day these readings were made:

A—very early plutei; *A'*—a very few late gastrulæ; *B*—early plutei; *B'*—very early plutei; *C* and *C'*—no difference apparent. A few late blastulæ, but mostly early plutei in both.

Conclusion.—A definite retardation was noted in the cultures containing extract at the early blastula stage and afterwards.

Series 53.

Extracts made up in alcohol (C_2H_5OH) must be used in small amounts, or else the alcohol must be a very weak solution, for if the concentration of alcohol in the cultures reaches three or four per cent. so many deaths occur in the blastula stage that comparison with the controls is difficult. Five series of experiments proved unsuccessful on this account.

In the experiment outlined below the extract was made up in 15 per cent. alcohol, and allowed to remain in the tightly corked tube for 24 hours before using.

TABLE XI.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c.	10 c.c.	Blastula	21.1° C.	8.2
A'.....	50 c.c.	10 c.c. of 15 per cent. alcohol	—	"	"
B.....	50 c.c.	3 c.c.	Blastula	"	"
B'.....	50 c.c.	3 c.c. of 15 per cent. alcohol	—	"	"
C.....	50 c.c.	1.5 c.c.	Blastula	"	"
C'.....	50 c.c.	1.5 c.c. of 15 per cent. alcohol	—	"	"

Later Development and Fate.—Very irregular cleavage in *A* and *A'*. No apparent difference between *B* and *B'*, *C* and *C'* at the blastula stage.

Later: *C'*—many normal plutei.

C—a few normal plutei.

B and *B'*—*B* very slightly retarded, with fewer and smaller plutei.

A and *A'*—as *B* and *B'*, with greater retardation in *A* than in *B*.

Conclusion.—Since alcohol, even in very weak concentrations, has such a powerful effect on the developing eggs, no very definite conclusion can be drawn from this series of experiments.

Series 63.

In the table below are given some observations made in sea water whose OH concentration had been raised by the addition of 0.8 c.c. $N/10$ NaOH to 50 c.c. sea water. This experiment was also designed as a check upon the experiments in which normal sea water was used. Loeb ('98) brought out the fact that by allowing eggs, fertilized normally, to develop up to the blastula stage, and then dividing the eggs into three lots, to one of which NaOH is added in the proportion of 1.76 c.c. $N/10$ NaOH to 100 c.c. of sea water, a second to which the same amount of $N/10$ HCl is added, and the third for the control, at a given time the bowl containing NaOH shows complete plutei, the HCl culture shows late gastrulæ with a few short-armed plutei, while the control contains many plutei in various stages, and some late gastrulæ. In the report of this experiment Loeb attributed this result to an increased rate of oxidation produced by the OH-ion.[17] Now, on this hypothesis, if the retardation in the development of the eggs due to the presence of the egg extract is a slowing down of the oxidation rate, then presumably the NaOH should neutralize, or antagonize this effect, and we should expect a rate of development more nearly normal.

The experiments proved that just the reverse is true, however, for the effect of the NaOH was not to neutralize, but rather to add to the effect of the extract, since it can be seen by glancing at the table that, if anything, in the culture containing NaOH plus extract, there was a slight retardation in the early cleavage rates compared with the NaOH control. A more marked degree of retardation was shown in the development from the blastula to the pluteus.

This result is in entire agreement with the later work of Loeb.[18] In his book he says that although the rate of development of *Arbacia* can be retarded by the addition of acids to the sea water, he has not succeeded in showing that the rate of development in *Arbacia* eggs can be accelerated by the use of hydroxyl-ions in the sea water. Glaser found that this latter

[17] Glaser, Otto, "Qual. Analysis of Egg Secretions and Extracts of *Arbacia* and *Asterias*," BIOL. BULL., Vol. XXVI., No. 6, June, 1914.

[18] Loeb, Jacques, "Artificial Parthenogenesis and Fertilization," Chicago, 1913.

statement seemed to hold good for the early cleavages, since there was no definite increase in rate observed there, but that the rate of development from blastula to pluteus was accelerated. It would appear, however, that from Loeb's account of his experiments, the acceleration mentioned in the first paper was not observed until after the first day, so that there is after all no disagreement between the results obtained by these two investigators.

TABLE XII.

Culture.	Amount of Sea Water.	Amt. of Extract.	Amount of *N*/10 NaOH.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A...	50 c.c.	3 c.c.	1.75 c.c.	Blastula	21.9° C.	8.2	57	70	76
A'..	53 c.c.	—	1.75 c.c.		"	"	48	54	71
A''..	54.75 c.c.	—	—		"	"	40	58	70
B...	50 c.c.	1.5 c.c.	1.75 c.c.	"	"	"	53	64	72
B'..	51.5 c.c.	—	1.75 c.c.		"	"	50	55	69
B''..	53.25 c.c.	—	—		"	"	43	53	68
C...	50 c.c.	0.5 c.c.	1.75 c.c.	"	"	"	49	54	67
C'...	50.5 c.c.	—	1.75 c.c.		"	"	51	57	64
C''..	52.25 c.c.	—	—		"	"	45	51	66

Note.—The time recorded under the cleavage rate is the number of minutes between insemination and the first appearance of the stages indicated.

Later Development and Fate.—It is evident that there is only a very slight retardation, if any, in the cultures containing the extract in small amounts during the early cleavages.

At a later reading *A* was found to contain non-motile blastulæ, *A'* motile blastulæ. In *B* and *B'*, *C* and *C'* no considerable difference was observed, except for the fact that in *B'* the blastulæ appeared to be swimming more vigorously than in *B*.

Still later, the reading showed the late gastrulæ in *A* dead while in *A'* were found some plutei of varying abnormality.

B—a few plutei, varying somewhat from the normal.

B'—flourishing plutei, varying somewhat from the normal.

C—as *B*; *C'* as *B'*.

The controls in sea water showed more rapid and normal development at all stages.

Conclusion.—A very slight retardation was produced by the use of the extract in the early cleavage stages. This effect was more evident from the blastula to the pluteus.

Experiments were also made using glycerine and chloroform for solvents, but since chloroform appeared to be very difficult to use, and since glycerine even in very small concentrations caused a high degree of mortality in the cultures, no further work was done at this point.

Analysis and Discussion of Results Obtained.

The end result of the investigations described in this report is that the larvæ of *Arbacia punctulata,* and probably of *Asterias forbesii,* contain substances which, when extracted in sea water or other solvents, *and when present in sufficient concentration,* retard the development of the eggs of the same species.

This retardation was shown in most cases only in a slight degree in the early cleavages, but more markedly in the later stages, especially at the early non-motile blastula stage. There would appear, then, to be certain periods at which the egg is more susceptible to the action of the extracts than at other times.

It is interesting to note in this connection that Lyon ('02) showed that eggs placed in a weak KCN solution gradually lose their resistance to the KCN as development proceeds. At the same time, there appeared to be certain periods at which the eggs were more susceptible to the poison than at other periods.

Child studied the effects of various chemical agents upon the egg of the sea-urchin to prove the existence of axial metabolic gradients as fundamental factors in the development of this form. While it was not at all proposed to attempt in these investigations a corroboration of Child's work, results in the controls in which the agents used as solvents for the larval extracts were the same as those which Child used, were similar, and many of the abnormal types which he described were noted.

Child[19] showed that the effect of KCN, C_2H_5OH, NH_4OH, NaOH, HCl, and CH_3COOH was a differential inhibition manifesting itself in various deviations from the normal course of

[18a] Lyon, E. P., "Effects of Potassium Cyanide and of Lack of Oxygen Upon the Fertilized Eggs and the Embryos of the Sea-urchin (*Arbacia punctulata*)." *Am. Journ. Physiol.* Vol. VII., pp. 56–75, 1902.

[19] Child, "Larval Development in the Sea Urchin," *Journ. of Morph.*, Vol. XXVIII., 1916–17.

development, as, for example, the variations of the angle of divergence between the arms, the approach of the lateral parts to the median line, and in extreme cases fusion, and many differences in the proportions of the larvæ.

Child also endeavored to show that differential acclimation and recovery may take place, resulting in wide-angled plutei, and an increase in the size of the oral lobe with over-development of the anterior and median regions as compared with the posterior and lateral regions. No specificity in the different form changes produced by the different agents used was noted.

In the experiments described here, also, the deviations from the normal form of the embryos produced by the extracts were so varied that there seems to be no basis for claiming any specificity of action. All the evidence points not toward a qualitative, but a quantitative action of the extract as far as the normal processes of growth and development are involved.

Although it is impossible at this point to state whether or not the different extracts all produce the same effect on the developing *Arbacia* egg, the fact which is to be emphasized is that, however they act, or whatever processes of the egg are chiefly involved, the general effect of the extracts when present in sufficient concentrations is to retard or inhibit the fundamental metabolic processes in some way.

There is a definite normal rate of development for the eggs of each animal, yet this rate may be changed by various conditions of environment. The two most common modifying causes are a change in oxidation (which may be due to a variety of causes) and a change in temperature. Among others, Stockard[20] has shown recently in many experiments that a very wide range in the decrease of developmental rate is very easily brought about by even a slight change in the surrounding temperature or a reduction in the oxygen supply. He has also contended that a normal continuous development may be modified into a discontinuous one by stopping its course during a very early stage.

Now many variations from the normal were found to be produced by the use of the extracts in certain concentrations. Some

[20] Stockard, C. R., "Developmental Rate and Structural Expression," *Amer. Journ. of Anat.*, Vol. XXVIII., No. 2, January 15, 1921.

of these were slight: others were more evident, as, for example, the exogastrulæ, noted in Table II. Their appearance may indicate an arrest of development at the gastrula stage or earlier; in fact, it was noticed that in most cases only after the larvæ had remained for a day at the gastrula stage, did the exogastrulæ appear. Some exogastrulæ, however, appeared after a slowing up of development from the blastula into the gastrula. The further records of experiments planned may render a more definite position on this point possible.

As far as temperature was concerned in carrying out these experiments, it would seem that the comparatively small variation from day to day noted above in the discussion of methods, would scarcely be sufficient to cause the development of abnormal types. And furthermore, if by any chance slight changes in the temperature from day to day might have resulted in the development of abnormalities, these abnormalities should be present in the controls in as great numbers as in the extract cultures. Such was not the case in the investigations outlined in this report.

In considering the part which the extract may have played in altering the normal oxidations in the developing eggs, three possibilities are evident: the rate of oxidation may be increased—in which case it would seem more probable that there would be an acceleration rather than a retardation in the rate of development, or the rate of oxidation may be decreased by the action of substances in the extract upon the eggs, or yet again the rate of oxidation may be decreased simply because the extract itself contains substances which use up the oxygen in the water, and so deprive the eggs of their otherwise available supply. No facts can be brought forward at this time, however, to make any one, or two, or all of these possibilities appear to be the probable explanation of the phenomena under discussion.

It should be emphasized at this point that the retardation caused by the use of the extract results in arrests of development and the production of abnormal types very similar to those caused by the various chemical agents used, although it is not possible of course to state definitely that these result from disturbances of the same metabolic processes.

Another fact which should be emphasized is that there was very evidently a shorter length of life in cultures containing extract than in the controls. This lowered degree of vitality, or of resistance, often manifested itself in a slower rate of movement, particularly at the blastula stage. A possible explanation of this effect may be found in part in the increased danger of bacterial infection which the use of extract affords. The presence of the crushed, and disorganized protoplasm of the extract affords a splendid opportunity for the breeding of bacteria. Thus it must be remembered that in considering the causes of the retardation, and the malformations resulting from the use of the extract, the matter of bacterial infection and more especially the effects of the toxins produced by these bacteria should receive due emphasis. It should be stated, however, that the experiments in which boiled extract was used furnish some evidence, very slight though it may be, that something other than bacterial action is involved in the retardation and the malformation resulting from the use of the extracts.

Summary.

1. Extracts of *Arbacia* larvæ in the 128–256-cell stage, in the early and in the late blastula, gastrula, and pluteus stages, when present in a sufficiently high concentration, definitely retard the development of eggs of the same species.

If these extracts are used in very low concentrations, the retardation may well lie within the limits of experimental and observational error.

2. The retardation noted is manifested slightly in the early cleavage rates, and more markedly in the later stages of development.

3. Besides retarding development, these embryological extracts often cause cytolysis, arrests of development, and a very noticeable failure of the eggs to develop beyond the early non-motile blastula stage.

4. The very evident tendency of the eggs to stop at the blastula stage suggests that possibly this stage is a stage peculiarly susceptible to the extract and characterized by a general lack of resistance.

5. It is possible that extracts made from larvæ in a certain stage differ qualitatively from those made of larvæ of an earlier or a later stage, but we do not know that this is true, nor do we know that the retardation is associated with any modification of the rate of enzyme action, or permeability, or oxidation, or any specific process. Probably it depends upon the great complexity of the protoplasmic system, and the fact that no one part of that system may be altered considerably without disturbing the equilibrium of that whole system.

6. It is not possible to say at this point that the retardation caused by the extract affects the same metabolic processes as do the KCN, CH_3COOH, NaOH, etc., but the use of the extract results in arrests and retardations of development such as are apt to be caused by these chemical agents, and the various types of malformations resulting are often similar.

7. This work does not indicate, then, either the presence or the absence of formative stuffs, but shows that under the conditions given they do not appear. Two possibilities present themselves. If formative stuffs exist, they would seem to be unable to pass into solution in sea water, in lipoid-soluble substances, and in solvents which are able to dissolve carbohydrates. Therefore, if present, they appear to be complex in character and in close association with the protein molecule. The other possibility is that these hypothetical substances may go into solution in certain solvents, but yet may not be able to register any effect upon the developing eggs, because the cell walls of the latter may not be permeable to the formative stuffs in solution.

[1] Conklin, E. G., "Heredity and Environment," p. 185.

[2] Child and Bellamy, "Physiological Isolation by Low Temperature," *Bot. Gaz.*, Vol. LXX., 1920, p. 249–267.

[3] Carnegie Institution of Washington Year Book 1918, p. 55, D. T. MacDougall. Report from the Department of Botanical Research, p. 55.

[4] MacBride, "Textbook of Embryology," Vol. I., p. 526–528.

[5] Driesch, '00, "Die isolierten Blastomeren des Echineden-Keimes Archer," *Ent. Mech.*, Vol. X.

[6] Wilson, E. B., "Experimental Studies on Germinal Localization," *Journ. Exp. Zoölogy*, Vol. I., p. 197–269.

[7] Conklin, E. G., "Organ-forming Substances in the Eggs of Ascidians," BIOL. BULL., Vol. VIII., No. 4.

[8] Morgan, T. H., "Regeneration."

[9] Stockard, C. R., "Structure and Developmental Rate," *Journ. of Anat.*, Vol. XXVIII., No. 2, January 15, 1921, p. 260.

[10] Lillie, F. R., "The Reproduction of Sperm Iso-Agglutinins by Ova."

[11] Glaser, Otto, "A Qualitative Analysis of the Egg Secretions and Extracts of *Arbacia* and *Asterias*," BIOL. BULL., Vol. XXVI., No. 6, June, 1914.

[12] Glaser, Otto, "The Change in Volume of *Arbacia* and *Asterias* Eggs at Fertilization," BIOL. BULL., XXVI., 1914.

[13] Chambers, Robert, "Personal Communication."

[14] Glaser, Otto, "Qualitative Analysis of Egg Extracts and Secretions of *Arbacia* and *Asterias*," BIOL. BULL., XXVI., 1914.

[15] Fuchs, H. M., "The Action of Egg Secretions on the Fertilizing Power of Sperm," *Arch. für Ent. der Org.*, Vol. XL., 1914. p. 248.

[16] Conklin, E. G., "The Effects of Centrifugal Force on the Eggs of *Crepidula*," *Journ. Exp. Zoölogy*, Vol. XXII., No. 2, February, 1917.